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Effects of Fermented Soya Bean Supplements on Thyroid Profile and Serum Electrolytes of High Fat Diet-induced Type 2 Diabetes Mellitus in Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author NMS performed the experimental work, collected the data and prepared the manuscript. Author YT designed the study work, supervised the research work and managed the statistical analysis. Authors AM and AAUD supervised the research work. Author AA assisted with the experimental work. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the effects of fermented soya bean supplements on thyroid profile and serum electrolytes of high fat diet-induced type 2 diabetes in rabbits.
Twenty rabbits weighing between 1 kg – 1.5 kg were used for the study. Diabetes was induced by feeding the animals with high fat diet for eight weeks. Rabbits having fasting blood glucose levels of 7.2 mmol/L and above after the induction were used for the study. The animals were grouped into four groups of five rabbits each: Group 1 (diabetic control) received distilled water *ad libitum* for six weeks; Groups 2, 3 and 4 were fed with 12.5%, 25% and 50% fermented soya bean supplements respectively for six weeks. At the end of the treatment period, the rabbits were euthanized by cervical dislocation and blood samples were collected through cardiac puncture. The serum was

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extracted after clotting and centrifugation for the determination of thyroid profile and electrolyte levels. The results showed a significant decrease *($P \leq 0.05$)* in serum T_3 and T_4 concentrations, and a significant increase *($P \leq 0.05$)* in the serum TSH concentration in the groups fed with the supplements when compared with the diabetic control. Furthermore, as regards to the serum electrolytes concentrations there was a significant increase ($P \leq 0.05$) in sodium ion concentration in the group fed 12.5% soya bean supplement when compared with the control and a significant decrease ($P \leq 0.05$) in potassium ion concentrations in all the groups fed with the soya beans supplements, as compared to control. Chloride ion concentrations were significantly decreased ($P \leq 0.05$) in groups fed 25% and 50 % supplement as compared to control, while bicarbonate ion concentrations were significantly increased ($P \leq 0.05$) in groups fed with 25% and 50% supplement when compared with the control. In conclusion fermented soya bean supplementation suppressed thyroid activity and improved serum electrolyte concentrations; it may be used to alleviate hyperthyroidism and electrolyte disturbances in diabetes patients.

Keywords: Type 2 diabetes mellitus; fermented soya beans; high fat diet; thyroid profile; serum electrolytes.

1. INTRODUCTION

Thyroid diseases and diabetes mellitus are the two most common endocrine disorders encountered in clinical practice. They mutually influence each other and their link have long been reported. Thyroid hormone is a major regulator of metabolism and energy expenditure, and is directly involved in the control of insulin secretion and glucose homeostasis [1,2]. It has been shown to preserve beta cell viability and proliferation [3,4] and higher free triiodothyronine levels are specifically associated with improved insulin secretion in individuals with prediabetes [5]. Thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, whereas diabetes mellitus affect thyroid function tests to variable extents [6].

A deep underlying relation between diabetes mellitus and thyroid dysfunction has been reported [7], and so many studies have evidenced an array of complex link with biochemical, genetic, and hormonal malfunctions mirroring diabetes and thyroid pathophysiological association [7,8]. 5′ adenosine monophosphate-activated protein kinase (AMPK) is a central target for modulation of insulin sensitivity and feedback of thyroid hormones associated with appetite and energy expenditure [8]. A meta-analysis reported a frequency of 11% in thyroid dysfunction in the patients of diabetes mellitus [9]. Autoimmunity has been implicated to be the major cause of thyroid-dysfunction associated diabetes mellitus [10,11,12].

Diabetes mellitus (DM) is frequently associated with electrolyte abnormalities, mostly caused by impaired renal function, malabsorption syndromes, acid-base disorders and multi-drug regimens, which are often present in diabetics [13,14]. The electrolytes variability is as a result of the high concentration of glucose in the extracellular fluid and low concentration in the intracellular fluid. This leads to disturbance in the fine equilibrium of electrolytes balance between the intracellular and the extracellular fluids [15]. Normal range of rabbit serum electrolytes are: sodium (Na^+), 135-160 mmol/L; potassium (K^+), 3.3-5.8 mmol/L; chloride (Cl^-), 93-116 mmol/L and bicarbonate (HCO_3^-), 16-30 mmol/L [16,17].

Botanically, soya bean belongs to the order *Rosaceae*, family *Leguminosae*, the genus Glycine and the cultivar Glycine max. Soya bean represents an excellent source of high quality protein, with a low content in saturated fat, containing a great amount of dietary fibre. Its isoflavone content makes it singular among other legumes [18]. Soya beans contain a significant amount of phytochemicals, which include isoflavones, phytosterols, saponins and lecithins. It also contain soluble fibres, polysaccharides and proteins, which may act collectively along with the phytochemicals or through independent mechanisms to give unique health benefits [19–21]. Hence, this study aimed at evaluating the effects of fermented soya bean supplements on thyroid profile and serum electrolytes of high fat diet-induced type 2 diabetes mellitus in rabbits.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Fermented Soya Bean Supplements

Soya bean of the variety, TGX-1448-2E was purchased from Institute for Agricultural

Research (IAR), Ahmadu Bello University, Zaria. The seeds were washed and soaked in a plastic container for forty eight hours, with the water unchanged (fixed fermentation) [22]. After forty eight hours, the seeds were drained, air dried and ground into fine granules. The fermented soya beans was then constituted into 12.5%, 25% and 50% supplements by combining the appropriate percentage by weight with the animal feed (The 12.5% contained, 12.5% fermented soya bean and 87.5% animal feed; 25% contained, 25% fermented soya bean and 75% animal feed; 50% contained 50% fermented soya bean and 50% animal feed).

2.2 Experimental Animals

Twenty (20) rabbits of both sexes weighing between 1 kg – 1.5 kg of about five to eight weeks of age were used for the study. The animals were obtained from the animal house, Faculty of Veterinary Medicine, ABU, Zaria. They were kept in well aerated laboratory cages in the animal house, Department of Human Physiology, ABU, Zaria and left to acclimatize for two weeks before the commencement of the experiment. They were fed with Grower's mash from Vital Feeds Company Plc. Jos and water was provided *ad libitum*.

2.3 Experimental Induction of Diabetes Mellitus

The rabbits were handled in accordance with the principles guiding the use and handling of experimental animals, ABU, Zaria. The animals were fasted from feeds for 12-14 hours prior to commencement of the experiment, but allowed water *ad libitum*. Type 2 diabetes mellitus was induced by feeding the animals with a high fat diet (2% cholesterol, 20% groundnut mill and 10% groundnut oil) [23] for eight weeks. After eight weeks of feeding with the high fat diet, blood was collected from the marginal vein of the ear lobe of the rabbits and glucose levels were determined using the glucose oxidase method [24] with the aid of a digital glucometer. Rabbits having fasting blood glucose levels of 7.2 mmol/L (130 mg/dL) and above were selected for the study [25,26].

2.4 Experimental Design

After the induction of type 2 diabetes mellitus in the rabbits, the animals were randomly assigned into experimental and control groups of five (5) rabbits each, as follows;

Group 1: Diabetic positive control, were allowed access to distilled water *ad libitum* for six weeks.
Group 2: Diabetic rabbits were fed *ad libitum* with 12.5% fermented soya bean supplement for six weeks.
Group 3: Diabetic rabbits were fed *ad libitum* with 25% fermented soya bean supplement for six weeks.
Group 4: Diabetic rabbits were fed *ad libitum* with 50% fermented soya bean supplement for six weeks.

At the end of the six weeks treatment period, the rabbits were euthanized by cervical dislocation and blood samples were collected from the animals through cardiac puncture for the determination of thyroid profile and serum electrolytes. The blood was allowed to clot and the serum separated by centrifugation.

2.5 Biochemical Analysis

The sera collected was used for the determination of thyroid profile: triiodothyronine (T_3) according to the method of [27]; tetraiodothyronine (T_4), according to [28]; and thyroid stimulating hormone (TSH), according to [29].

Serum electrolytes: sodium ion (Na^+) and potassium ion (K^+) were estimated by flame photometry method [30]; bicarbonate ion (HCO_3^-) was determined by the titration method of [31]; and chloride ion (Cl^-) was estimated by method of [32].

2.6 Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. Statistical comparisons was performed by one way analysis of variance (ANOVA). The results were considered statistically significant if the $P \leq 0.05$.

3. RESULTS

3.1 Effect of Fermented Soya Bean Supplements on Thyroid Hormones Profile

The result of thyroid profile shown in Table 1 shows a statistically significant decrease ($p \leq 0.05$) in triiodothyronine (T_3) (1.54 ± 0.05 ng/mL, 1.40 ± 0.07 ng/mL and 1.62 ± 0.14 ng/mL) in the groups fed 12.5%, 25% and 50% fermented soya bean supplements respectively, when compared with the control group (2.10 ± 0.07 ng/mL). Thyroxine (T_4) was also significantly decreased ($P \leq 0.05$) to (68.60 ± 1.36 ng/mL, 61.40 ± 1.12 ng/mL and 63.80 ± 1.28 ng/mL) in the groups fed 12.5%, 25% and 50% fermented soya bean supplements respectively, when compared with the control (79.40±1.50 ng/mL). However, thyroid stimulating hormone (TSH) concentrations were significantly increased ($P \leq 0.05$) to (1.72 ± 0.06 ng/mL,1.66 ± 0.14 ng/mL and 1.30 ± 0.11 ng/mL) in the groups fed 12.5%, 25% and 50% fermented soya bean supplements respectively when compared with the control group (0.76 ± 0.09 ng/mL).

3.2 Effect of Fermented Soya Bean Supplements on Serum Electrolytes

The result of serum electrolytes shown in Table 2 shows a significant increase ($P \leq 0.05$) in sodium ion concentration in the group fed 12.5% fermented soya bean supplement (130.27 ± 6.70 mmol/L) when compared with the control group (86.91 ± 6.99 mmol/L). Potassium ion concentrations were significantly decreased ($P \leq 0.05$) in the groups fed 12.5%, 25% and 50% fermented soya bean supplements (6.49 ± 0.26 mmol/L, 4.32 ± 0.21 mmol/L and 6.34 ± 0.25 mmol/L respectively), when compared with the control group (11.2 ± 0.34 mmol/L). The result also showed a significant decrease ($P \leq 0.05$) in chloride ion concentration in the groups fed 25% and 50% fermented soya bean supplements (80.20 ± 0.20 mmol/L and 82.40 ± 0.4 mmol/L respectively), when compared with the control (92.40 ± 1.25 mmol/L). Lastly, bicarbonate ion concentrations were significantly increased ($P \leq 0.05$) in the groups fed 12.5% and 25% fermented soya bean supplements (39.20 ± 0.80 mmol/L and 36.80 ± 0.20 mmol/L) respectively, when compared with the control (27.00 ± 0.63 mmol/L).

4. DISCUSSION

The result of this study showed significant decrease in the serum concentrations of T_3, T_4 and significant increase in the serum concentration of TSH in all the treated groups, when compared with the untreated diabetic control group. Thyroid hormones are insulin antagonists. Both insulin and thyroid hormones are involved in cellular metabolism. Excess or deficit of any one of these hormones can result in functional derangement of the other. Diabetes mellitus has been known to impair thyroid

Table 1. Effect of fermented soya bean supplements on thyroid hormones profile of high fat diet– induced diabetic rabbits

Group	T_3 (ng/mL)	T_4 (ng/mL)	TSH (ng/mL)
Untreated diabetic	2.10 ± 0.07	79.40 ± 1.50	0.76 ± 0.09
DM + 12.5% SY	1.54 ± 0.05[a]	68.60 ± 1.36[a]	1.72 ± 0.06[a]
DM + 25% SY	1.40 ± 0.07[a]	61.40 ± 1.12[a]	1.66 ± 0.14[a]
DM + 50% SY	1.62 ± 0.14[a]	63.80 ± 1.28[a]	1.30 ± 0.11[a]

Values are expressed as mean ± SEM; n = 5.
a: Value considered statistically significant when compared with control group (p ≤ 0.05).
SEM = Standard error of mean.
SY – Fermented soya bean supplement
T_3 – Triiodothyronine; T_4 – Thyroxine;
TSH – Thyroid stimulating hormone; n = Number of animals

Table 2. Effect of fermented soya bean supplements on serum electrolytes of high fat diet–induced diabetic rabbits

Group	Sodium (Na^+) (mmol/L)	Potassium (K^+) (mmol/L)	Chloride (Cl^-) (mmol/L)	Bicarbonate (HCO_3^-) (mmol/L)
Untreated diabetic	86.91 ± 6.99	11.21± 0.34	92.40 ± 1.25	27.00 ± 0.63
DM + 12.5% SY	130.27 ± 6.70[a]	6.49 ± 0.26[a]	95.20 ± 1.02	39.20 ± 0.80[a]
DM + 25% SY	73.30 ± 5.08	4.32 ± 0.21[a]	80.20 ± 0.20[a]	36.80 ± 0.20[a]
DM + 50% SY	78.73 ± 10.93	6.34 ± 0.25[a]	82.40 ± 0.4[a]	28.20 ± 1.20

Values are expressed as mean ± SEM; n = 5.
a: Value considered statistically significant when compared with control group (p ≤ 0.05).
SEM = Standard error of mean.
SY – Fermented soya bean supplement
n = Number of animals

metabolism. At low insulin sensitivity, minor changes in TSH levels is associated with marked changes in lipid metabolism and thus, increased cardiovascular risk [33]. The observed decrease in serum levels of T_3 and T_4 in the groups treated with fermented soya bean supplements may be as a result of the effect of isoflavones, which are said to inhibit thyroid peroxidase enzyme activity, thereby reducing thyroid hormone levels which in turn decreases body metabolism and electrolyte levels [34]. These results of study indicate the protective effect of fermented soya bean supplement against increased levels of thyroid hormones. The increase in the concentration of TSH in the treated groups when compared with the untreated diabetic control group may be as a result of a negative feedback on the anterior pituitary gland by the decreased levels of T_3 and T_4, leading to increased secretion of the TSH from the anterior pituitary gland. It has been reported that genistein and daidzein administered subcutaneously induced changes in the angio-follicular units of the thyroid gland, causing reduced concentration of total thyroid hormones (TH) and increased concentration of thyroid stimulating hormone (TSH), in serum of orchidectomised middle-aged rats. Thyroglobulin and TH synthesis were also impaired by the soya bean components, while tissue availability of TH was increased in peripheral tissues of the orchidectomised middle-aged rats [35]. The genistein and daidzein present in the fermented soya beans are therefore, most likely responsible for the effects seen on the thyroid profile.

The result of the current study also showed higher concentrations of sodium (Na^+), potassium (K^+) and chloride (Cl^-) in the untreated diabetic control group when compared with the 25% and 50% fermented soya bean supplemented groups. The decrease were statistically significant (*P* ≤ 0.05) in the K^+ and Cl^- concentrations, and non-significant in the Na^+ concentration (which may be biologically relevant). The kidneys work to keep the electrolyte concentrations in the blood constant despite changes in the body. The serum concentrations of electrolytes are usually indicative of the renal functions or dysfunctions. Cellular membrane electrolyte transporter (Na^+-K^+- ATPase) dysfunction has been observed in diabetic subjects, secondary to hyperglycaemia [36], which leads to derangement in serum sodium and potassium in diabetics. The changes observed in the present study may be due to the effect of isoflavones, which are known to exert antioxidant effect, shown to be of benefit in the therapy of kidney diseases associated with insulin resistance. This may be by direct inhibition of the Na^+-K^+-Cl^- cotransporter system of the thick ascending limb of the loop of Henle [37]; thereby decreasing the reabsorption of these electrolytes; and their subsequent excretion from the kidney.

Metabolic acidosis is strongly linked with diabetes as a result of distorted anion gap. This leads to a deficit in HCO_3^- concentration, resulting from its massive utilization for buffering [15]. Our result showed a significant increase (*P* ≤ 0.05) in HCO_3^- concentration in the groups fed 12.5% and 25% fermented soya bean supplements when compared with the diabetic control group. The increase in HCO_3^- concentration indicates the ability of the fermented soya bean supplement to counteract metabolic acidosis and probably restore acid-base balance.

5. CONCLUSION

The results of the present study showed that fermented soya bean supplementation suppressed thyroid activity and improved serum electrolyte concentrations in high fat diet - induced type 2 diabetes mellitus in rabbits, and thus, may be used to alleviate hyperthyroidism and electrolyte disturbances in diabetic patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We declare that all the experiments were performed in accordance with the ethical guidelines and approval of the ethics committee for the use and handling of experimental animals, Ahmadu Bello University, Zaria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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